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EDUCATION

Elizabeth Njogu

GEIS function has been designed to include support of global surveillance, training, research, and response to emerging infectious disease threats. The work is designed to reduce global disease threats through: centralized coordination; improved preventive health programs and epidemiological capabilities; and enhanced involvement in treatment facilities and overseas laboratories.

Capacity development is an integral part of these GEIS activities. While basic infrastructure and continued technical and financial support are a vital part of the daily operations, education is one of the most important for long term benefit. It is the belief that while GEIS can support local scientists by the provision of infrastructure, education is a far greater benefit as the scientist is able to continue working with or without GEIS resources.

There are several areas where education is encouraged. These include: a two-month laboratory training program for undergraduate students, support of graduate education of several host nation scientists, and an outbreak investigation course. In-house training for staff members is also done on a continuous basis in various aspects such as Quality Control and Assurance, Good Laboratory Practices, refresher courses on SOPs, and workshops.

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The benefits of this education are clear. Well trained personnel are better able to perform the duties that make the GEIS activities a continued success. Both local and foreign students who come for the laboratory training programs are given a better view of what goes on in a research facility and they are encouraged to look into working in this field or to taking the acquired knowledge to the outside world where it can be more beneficial. By training health care providers in outbreak investigation laboratory staff are able to recognize disease outbreaks early, as a result the number of persons who become ill or die can be limited and precious national resources saved for other health problems.

While it is difficult to document the exact benefits of the education programs undertaken by GEIS, The GEIS Gazette will feature some of the studies that are currently being undertaken by graduate students from different sections. A small part within this broad area, the Masters and PhD studies are a vital part in the continued fight against infectious diseases in Kenya and the world.

CHARACTERISATION OF GROUP B *STREPTOCOCCUS* (GBS) IN PELVIC INFLAMMATORY DISEASE IN HIV POSITIVE AND NEGATIVE WOMEN IN NAIROBI KENYA

Bonventure Juma

Pelvic inflammatory disease (PID) comprises endometritis, salpingitis and abscesses. The infection can result in major health problems such as tubal factor infertility, ectopic pregnancy, abortion, still birth, chronic pelvic pain and fever. PID plays a major role in infertility in women of reproductive age. On the other hand infection caused by Human Immunodeficiency Virus (HIV) causes major health problems such as rapid reduction in the protective capacity of the body's immune system and subsequent opportunistic infections such as protozoal, fungal, bacterial, and viral infections that culminate in high death rates in all age groups. Globally HIV/AIDS is one of the major causes of death and may be responsible for more than eight million deaths per year in Africa alone.

PID plays a major role in infertility in women.

The aim of the this study was to characterize *Streptococcus agalactiae* isolated from the women seen at the Obstetric and Gynecology clinics at Kenyatta National Hospital and the Nairobi City Council Special Treatment Clinic between 2003 and 2004. The age of women participants varied between 18 to 40 years. During the study a total of 150 women with laparascopically confirmed PID or Bacterial Vaginosis were admitted at the Gynecology Ward. A total of 120 biopsies and 30 high vaginal swabs were obtained and cultured. The cultures yielded different micro organisms of which 32.1% were Group B Streptococcus.

The two tests (CAMP and Hippurate) which are relied on in the screening of GBS were validated for sensitivity and specificity. A total of fourteen GBS biotypes were obtained. Some were site specific, others specific to HIV serostatus while others were evenly distributed. Antibiotic susceptibility testing was carried out using both disk-diffusion and MIC methods according to NCCLS (2002) guidelines. The bacteria were shown to be resistant to a wide range of antibiotics including: Gentamicin (1OO %), Tetracycline (91%), Oxacillin (82%), Chloramphenical (81%), Erythromycin (54.5%), and Penicillin (9%).

There was a significant correlation between susceptibility profiles determined by disk and minimum inhibition concentration assays and the multiplex PCR assay for the simultaneous detection of the antibiotic resistance genes (p=0.0144). Significantly more GBS were found in HIV positive than in HIV negative women (P= 0.0016). There was also a significant association between HIV and PID (P= 0.0120). This is so because patients with HIV infection may have increased risk of PID, although several potential biases may distort the recognition and incidence. PID on the other hand can also facilitate the acquisition of HIV due to gradual reduction of hydrogen peroxide producing *Lactobacillus spp*. (Klebanoff , 1991; Klebanoff et al., 1991).

Hydrogen peroxide producing *Lactobacillus* are cidal to HIV virus (Cohen, 1995). Hydrogen peroxide producing lactobacilli also produce lactic acid which is toxic to a variety of micro organisms (Whittenburry, 1964; Eschenbach, 1989; Klebanoff *et al.,* 1991). *Lactobacillus spp* act differently to different micro organisms. For example; *Lactobacillus acidophilus* is the first line of defense against invaders and opportunistic organisms such as yeast and prevention of the pathogen from accessing the lining of the vaginal wall while *Lactobacillus casei* protects against infections caused by *Listeria* spp. *Lactobacillus plantarum* is an important tool in antimicrobial defense.

GBS is a major problem both for the fetus, expectant mothers and women with PID. It is a commensal flora in the female genitalia and the rectum. Infants acquire it *in utero* by ascending route through rupture or intact membranes or during the birth process. Expectant mothers should therefore be thoroughly screened for GBS during the antenatal care from the 35th to 37th weeks of gestation to rescue both the mother and the fetus. Those found positive for GBS should be put on medication following the WHO recommendations of 1996. From our findings GBS is shown to be very common in women (25%) and it is a multi-drug resistant; thus a matter of public concern.

In Vitro Malaria Drug Screening Laboratory

Pamela Liyala

The in vitro malaria drug screening laboratory is a section of the GEIS Malaria laboratory, Nairobi. Anti-malarial drug resistance has emerged as one of the greatest challenge facing malaria control today. Our major objective is to provide proof-of-concept data on malaria drug resistance patterns in Kenya. This is important because recommended treatment regimens should be tailored specifically to a given region based on resistance patterns found in that area. Additionally, we also support new anti-malarial drug development projects by screening potential anti-malarial drug compounds from plant extracts in collaboration with the University of Nairobi.

Blood samples with *Plasmodium falciparum* parasites from the GEIS satellite sites are transported to the Drug Screening laboratory in Nairobi and cultured in vitro. The GEIS program is involved in testing a panel of 15 anti-malarial drugs. These include: chloroquine, mefloquine, quinine, artemisinin, halofantrine, doxcycycline, amodiaquine, primaquine, atovaquone, tafenoquine, dapsone, proguanil, sulfadoxine, chlorocycloguanil and pyrimethamine. These drugs are dissolved and coated in micro-titer plates. After the parasites have adapted in culture, (it takes approximately two weeks to have the cultures adapted) they are exposed to the anti-malarial drugs for 24 hours (folate drugs) and 48 hours (antifolate drugs) after which they are labeled with tritiated hypoxanthine for further 18 hours.

Resistant isolates are those that incorporate tritiated hypoxanthine as compared to standard control parasite strains W2 (resistant strain) and D6 (sensitive strain). This information contributes to a growing body of evidence that can be used to determine the malaria treatment policy in this country. This lab is run by Josphat Mwangi, Julia Wangui, Hosea Akala and Pamela Liyala.

Comoros Outbreak Investigations

Victor Otieno

In mid-January 2005, public health authorities in Comoros were alerted to suspected cases of Dengue Fever in the Comoros. Affected patients presented with fever, severe arthralgia, rash, headache, and general malaise. To address this public health problem, the Comorian government through the WHO country office requested for assistance from the regional office, which called upon the CVR/KEMRI laboratory to assist with the diagnosis.

Serum specimens from 25 clinical cases received at USAMRU-K/KEMRI Laboratory were tested for alphavirus antibodies and nucleic acid. Nine of 25 were positive for anti-CHIK virus IgM antibodies. Six of 11 specimens tested were positive for alphavirus nucleic acid by reverse transcriptase-polymerase chain reaction (RT-PCR); genomic sequencing of the PCR bands confirmed CHIK virus as the etiologic agent.

What is CHIK? CHIK is a synonym for Chikungunya virus. The word "chikungunya", Swahili for "that which contorts or bends up," refers to the contorted posture of patients afflicted with the arthralgia (sever joint pain), a common symptom of Chikungunya Fever. It belongs to the Togaviridae family (formerly Group A Arboviruses), Genus Alphavirus which are spherical, enveloped virions, 60nm in diameter, with a single stranded, positive-sense RNA genome.

CHIK is self limiting febrile viral disease. The infection is characterized by the sudden onset of high fever, chills, headache, nausea, vomiting, arthralgia (joint pain), and rash. The maculopapular rash mainly involves the trunk and the limbs, but the face, palms, and soles may also show lesions. Mild haemorrhaging may be present especially in children. Unapparent infections are common and immunity is long lasting. Illness tends to last 3-10 days with the arthralgia remaining a problem for weeks to several months after the initial phase. The incubation period is usually 2-4 days.

Chikungunya virus continues to cause major

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epidemics in the tropical region of Asia and Africa since it was first recognized in epidemic form in East Africa in 1952. The host are humans, primates, other mammals and birds.

Chikungunya is transmitted by a bite from an infective mosquito of *Aedes spp* (*Ae. aegptyi, Ae. africanus*) and *Mansoni spp.* and epidemics are sustained by human-mosquito-human transmission. This vector is similar to the one responsible for the spread of Dengue Fever. Although the vector is similar, in contrast to Dengue, Chikungunya is characterized by a briefer febrile episode, persistent arthralgia in some cases, and by the absence of fatalities. While there is no cure for the disease at the present, prevention looks at controlling mosquitoes and avoiding mosquito bites. Rest is recommended to alleviate acute symptoms, while mild exercise is recommended to improve arthralgia. Heavy exercise, however, may exacerbate arthritic symptoms. 🗣

Protocol Update: Adjusted

Elizabeth Njogu

The time line on the Enteric Protocol has been adjusted to make allowances for some alterations. Minor modifications to the protocol have been completed and ensure a more comprehensive study of all the samples coming in.

The exact starting date will be communicated to all those concerned in due time.

Training is complete in all five GEIS sites as well as the Embassy staff participating in that section of the surveillance. In-house training and refresher courses for the laboratory staff have also been running and included both dry and wet runs through all aspects of protocol related Standard Operating Procedures (SOPs)

Led by Willie Sang, the Enteric Department team consisting of Valerie Oundo, Bonventure Juma, Paulomi Patel and Elizabeth Njogu practiced the procedures outlined on collected field samples.

The run through included the steps in the protocol and was designed to give the personnel a reminder of the processes as well as work through any problems that may arise while the protocol is running. Of most importance was to ascertain that all the required supplies were available to ensure smooth running once the protocol begins and samples start coming in.

With the recent outbreaks of diarrhea in different parts of the country the start of this surveillance protocol is very important and will possible help in the discovery and control of possible sources of the causative agents. 🗣

IDENTIFICATION OF INTERACTING PARTNERS OF TWO *PLASMODIUM FALCIPARUM* CYCLIN-DEPENDENT KINASES: PFPK6 AND PFMRK, USING THE BACTERIAL TWO HYBRID SYSTEM.

Fred Eyase

At present about 100 countries are considered malaria endemic. Almost half of the affected population is in sub-Saharan Africa. The malaria problem persists in spite of more than a century of efforts to eradicate or control it. One quarter of the world's population is at risk of infection with malaria. For the most part this disease has gone unnoticed in the developed regions of the world.

This disease is caused by the infection with intracellular parasites of the Genus Plasmodium that are transmitted by female Anopheles mosquitoes. Of all the species of Plasmodium that infect humans, *Plasmodium falciparum* is the most fatal. The incidence of malaria is estimated to be 300-500 million clinical cases each year mostly caused by *P. falciparum*. 90% of these occur in Africa with mortality at approximately 2.7 million cases each year. The mortalities are highest in sub-Saharan Africa where children under 5 years of age account for 75% of all death. Fatality rates of 10-30% have been reported among children referred to hospital with severe malaria, although this rate is even higher in rural and remote areas where patients have restricted access to adequate treatment.

Resistance of *P. falciparum* to chloroquine is now common in practically all malaria endemic countries of Africa especially in East Africa, thus posing increasing problems for the provision of suitable treatment. It is therefore imperative to look at the possibility of new drug targets to avoid running out of anti-malarial chemotherapeutic options.

Cyclin-Dependent Kinases (CDKs) have already been selected as attractive anti-cancer drug targets and this is being extrapolated to other diseases. This study focused on two *Plasmodium falciparum* CDKs namely PfPk6 and Pfmrk. Since the *P. falciparum* cell cycle is complex and quite different from the traditional eukaryotic cell cycle model, it would be helpful to study and understand the mechanisms of this unique developmental cycle by identifying the interacting partners of the two plasmodial CDKs.

To determine which other proteins interact with the said CDKs, a bacterial two-hybrid system was used. The two-hybrid system is a fast and efficient method for detecting protein-protein interactions in vivo. The system allows the identification of genes encoding proteins that interact with a target protein. It involves the cloning of a known parasite gene into a bacterium and interacting it with an unknown parasite gene cloned in the same bacterium (hence the name two-hybrid).

Using this system we have been able to isolate six different proteins that interact with the two CDKs. Analysis of these proteins indicates that three are involved in parasite DNA replication and processing. Two are involved in red blood cell adhesion to the host microvasculature and one of them is an adapter molecule which acts as a platform on which other proteins interact to form complexes. Furthermore, these proteins contain signatures that suggest their involvement in the integration of the cell cycle to the other cellular physiological functions.

Resistance of Plasmodium falciparum to chloroquine is now common posing increasing problems for the provision of suitable treatment.

One of the signatures found to be heavily present in majority of the identified proteins is already under study as a chemotherapeutic target. The Casein Kinase 1 is an enzyme that performs many diverse functions in all living systems. It functions by interacting with other proteins. Signatures which act as docking sites for CK1 have been seen in the identified proteins. The importance of this new information is that Purvalanol B which is a competitive inhibitor of Casein Kinase 1 has been found to be lethal to *Plasmodium falciparum* in vitro and yet it shows no effect on mammalian cells.

Studies from natural product chemistry suggest that natural products with anti-parasitic activity function by complexing with key parasite proteins thereby inhibiting protein-protein interactions. More studies will be required to determine the extent of the chemotherapeutic importance of these proteins.

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